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# Rapid Increase in Pertactin-deficient *Bordetella pertussis* Isolates, Australia

## Technical Appendix

### Characteristics of Pertactin-deficient *Bordetella pertussis* Isolates, Australia, 2008–2012

Technical Appendix Table. Characteristics of perlactin-negative isolates of *Bordetella pertussis* from 5 states of Australia, 2008–2012*

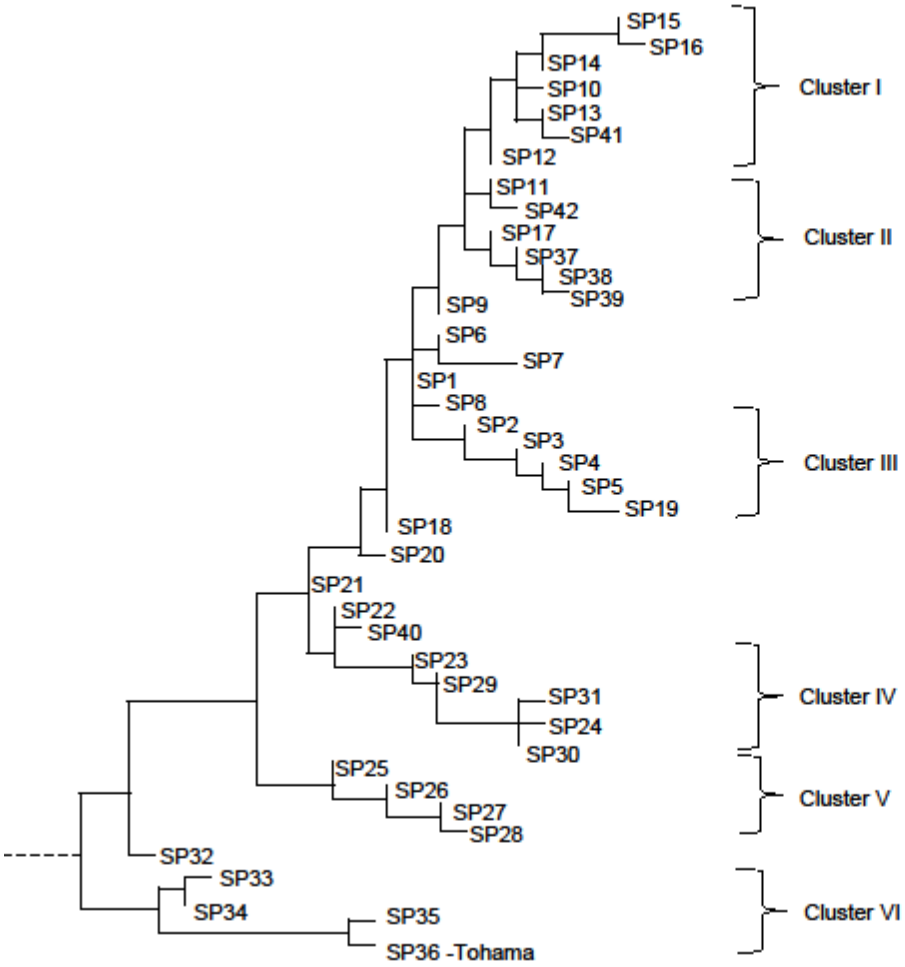
| Strain | Year | SP | MLVA† | Disruption‡ | *prn* | *fim* | *Ptx* |
|---|---|---|---|---|---|---|---|
| South Australia | | | | | | | |
| L1277 | 2008 | 14 | New 2 (27) | No ISE | *prn2* | A | *ptxP3* |
| L1283 | 2009 | 14 | 27 | IS*481*R | *prn2* | A | *ptxP3* |
| L1287 | 2009 | 14 | New 2 (27) | No ISE | *prn2* | A | *ptxP3* |
| L1290 | 2009 | 14 | 27 | IS*481*R | *prn2* | A | *ptxP3* |
| L1294 | 2009 | 14 | 27 | IS*481*R | *prn2* | A | *ptxP3* |
| L1295 | 2009 | 13 | 27 | No ISE | *prn2* | B | *ptxP3* |
| L1297 | 2009 | 14 | 27 | IS*481*R | *prn2* | A | *ptxP3* |
| L1308 | 2009 | 13 | 28 | No ISE | *prn2* | B | *ptxP3* |
| L1314 | 2009 | 13 | New 14 (214) | No ISE | *prn2* | A | *ptxP3* |
| L1326 | 2008 | 13 | New 2 (27) | No ISE | *prn2* | A | *ptxP3* |
| Victoria | | | | | | | |
| L1333 | 2009 | 14 | New 7 (72) | IS*481*F | *prn2* | B | *ptxP3* |
| L1352 | 2010 | 14 | 18 | No ISE | *prn2* | B | *ptxP3* |
| Western Australia | | | | | | | |
| L1361 | 2009 | 13 | New 3 (214) | IS*481*R | *prn2* | A | *ptxP3* |
| L1371 | 2009 | 13 | New 3 (214) | IS*481*R | *prn2* | A | *ptxP3* |
| L1378 | 2010 | 18 | New 8 | No ISE | *prn1* | A | Not *ptxP3* |
| L1749 | 2011 | 13 | 27 | IS*481*R | *prn2* | A | *ptxP3* |
| L1751 | 2011 | 13 | 27 | IS*481*R | *prn2* | A | *ptxP3* |
| L1752 | 2011 | 13 | 27 | IS*481*F | *prn2* | A | *ptxP3* |
| L1753 | 2011 | 13 | 27 | IS*481*F | *prn2* | A | *ptxP3* |
| L1756 | 2011 | 13 | 27 | IS*481*R | *prn2* | A | *ptxP3* |
| L1757 | 2011 | 14 | 18 | No ISE | *prn2* | B | *ptxP3* |
| L1759 | 2011 | 13 | 27 | IS*481*R | *prn2* | A | *ptxP3* |
| L1762 | 2011 | 14 | 18 | No ISE | *prn2* | B | *ptxP3* |
| L1763 | 2011 | 13 | 27 | IS*481*R | *prn2* | A | *ptxP3* |
| L1764 | 2011 | 13 | 27 | IS*481*F | *prn2* | A | *ptxP3* |
| L1766 | 2011 | 13 | 27 | IS*481*R | *prn2* | A | *ptxP3* |
| L1767 | 2011 | 13 | 27 | IS*481*R | *prn2* | A | *ptxP3* |
| L1768 | 2011 | 13 | 27 | IS*481*R | *prn2* | A | *ptxP3* |
| L1769 | 2011 | 13 | 27 | IS*481*R | *prn2* | A | *ptxP3* |
| L1771 | 2011 | 14 | 18 | No ISE | *prn2* | B | *ptxP3* |
| L1772 | 2012 | 13 | 27 | IS*481*R | *prn2* | A | *ptxP3* |
| L1773 | 2012 | 13 | 27 | IS*481*R | *prn2* | A | *ptxP3* |
| L1775 | 2012 | 13 | 27 | IS*481*R | *prn2* | A | *ptxP3* |
| L1776 | 2012 | 13 | 27 | IS*481*R | *prn2* | A | *ptxP3* |
| L1777 | 2012 | 13 | 27 | IS*481*R | *prn2* | A | *ptxP3* |
| L1778 | 2012 | 13 | 27 | IS*481*R | *prn2* | A | *ptxP3* |
| L1779 | 2012 | 13 | 27 | IS*481*R | *prn2* | A | *ptxP3* |
| L1782 | 2012 | 13 | 27 | IS*481*R | *prn2* | A | *ptxP3* |
| L1783 | 2012 | 13 | 27 | IS*481*R | *prn2* | A | *ptxP3* |
| L1784 | 2012 | 13 | 27 | IS*481*R | *prn2* | A | *ptxP3* |
| L1786 | 2012 | 13 | 27 | IS*481*R | *prn2* | A | *ptxP3* |
| L1787 | 2012 | 13 | 27 | IS*481*R | *prn2* | A | *ptxP3* |
| L1788 | 2012 | 13 | 27 | IS*481*R | *prn2* | A | *ptxP3* |
| L1789 | 2012 | 13 | 27 | IS*481*F | *prn2* | A | *ptxP3* |
| L1790 | 2012 | 13 | 27 | IS*481*R | *prn2* | A | *ptxP3* |
| L1791 | 2012 | 13 | 27 | IS*481*R | *prn2* | A | *ptxP3* |
| L1793 | 2012 | 13 | 27 | IS*481*F | *prn2* | A | *ptxP3* |
| L1795 | 2012 | 13 | New 3 (214) | IS*481*R | *prn2* | A | *ptxP3* |
| L1796 | 2012 | 13 | 27 | IS*481*R | *prn2* | A | *ptxP3* |
| L1797 | 2012 | 13 | 27 | IS*481*F | *prn2* | A | *ptxP3* |
| L1798 | 2012 | 13 | 27 | IS*481*R | *prn2* | A | *ptxP3* |
| New South Wales | | | | | | | |

| Strain | Year | SP | MLVA† | Disruption‡ | *prn* | *fim* | *Ptx* |
|---|---|---|---|---|---|---|---|
| L1421 | 2010 | 13 | New 19 (114) | IS*1002* | *prn2* | A | *ptxP3* |
| L1422 | 2010 | 13 | New 19 (114) | IS*1002* | *prn2* | A | *ptxP3* |
| L1424 | 2010 | 16 | New 19 (114) | IS*481*R | *prn2* | B | *ptxP3* |
| L1425 | 2010 | 16 | New 19 (114) | IS*481*R | *prn2* | B | *ptxP3* |
| L1429 | 2010 | 16 | New 19 (114) | IS*481*R | *prn2* | B | *ptxP3* |
| L1432 | 2010 | 16 | New 22 (114) | IS*481*R | *prn2* | B | *ptxP3* |
| L1492 | 2011 | 16 | 27 | IS*481*R | *prn2* | B | *ptxP3* |
| L1493 | 2011 | 13 | 27 | IS*481*R | *prn2* | A | *ptxP3* |
| L1495 | 2011 | 16 | 27 | IS*481*F | *prn2* | B | *ptxP3* |
| L1496 | 2011 | 13 | 27 | IS*1002* | *prn2* | A | *ptxP3* |
| L1498 | 2011 | 16 | 27 | IS*481*R | *prn2* | B | *ptxP3* |
| L1499 | 2011 | 13 | 27 | IS*481*F | *prn2* | A | *ptxP3* |
| L1501 | 2011 | 13 | NA | IS*1002* | *prn2* | A | *ptxP3* |
| L1502 | 2011 | 13 | 27 | HPT-G | *prn2* | A | *ptxP3* |
| L1503 | 2011 | 13 | 27 | IS*481*R | *prn2* | A | *ptxP3* |
| L1504 | 2011 | 13 | 27 | IS*1002* | *prn2* | A | *ptxP3* |
| L1505 | 2011 | 16 | New 23 | IS*481*R | *prn2* | A | *ptxP3* |
| L1506 | 2011 | 16 | 27 | IS*481*R | *prn2* | B | *ptxP3* |
| L1507 | 2011 | 13 | 27 | IS*481*R | *prn2* | A | *ptxP3* |
| L1508 | 2011 | 16 | 27 | IS*481*R | *prn2* | B | *ptxP3* |
| L1529 | 2011 | 13 | 27 | IS*481*R | *prn2* | B | *ptxP3* |
| L1532 | 2011 | 13 | New 2 (27) | No ISE | *prn2* | A | *ptxP3* |
| L1554 | 2011 | 13 | 27 | IS*481*R | *prn2* | A | *ptxP3* |
| L1658 | 2012 | 13 | 27 | IS*1002* | *prn2* | A | *ptxP3* |
| L1659 | 2012 | 14 | 18 | No ISE | *prn2* | B | *ptxP3* |
| L1661 | 2012 | 13 | 27 | IS*481*R | *prn2* | A | *ptxP3* |
| L1662 | 2012 | 13 | 27 | IS*481*R | *prn2* | A | *ptxP3* |
| L1663 | 2012 | 13 | 27 | IS*481*F | *prn2* | A | *ptxP3* |
| L1664 | 2012 | 16 | 27 | IS*481*R | *prn2* | B | *ptxP3* |
| L1665 | 2012 | 16 | 27 | IS*481*R | *prn2* | B | *ptxP3* |
| Queensland | | | | | | | |
| L1512 | 2010 | 13 | 27 | IS*481*F | *prn2* | A | *ptxP3* |
| L1513 | 2010 | 13 | 22 | No ISE | *prn2* | A | *ptxP3* |
| L1514 | 2010 | 13 | 27 | IS*481*R | *prn2* | A | *ptxP3* |
| L1515 | 2010 | 13 | 27 | IS*481*R | *prn2* | A | *ptxP3* |
| L1516 | 2010 | 13 | 27 | IS*481*R | *prn2* | A | *ptxP3* |
| L1519 | 2010 | 13 | 27 | IS*481*F | *prn2* | A | *ptxP3* |
| L1526 | 2011 | 13 | 27 | IS*481*F | *prn2* | A | *ptxP3* |
| L1534 | 2011 | 13 | 27 | IS*481*R | *prn2* | A | *ptxP3* |
| L1536 | 2011 | 13 | New 2 (27) | No ISE | *prn2* | A | *ptxP3* |
| L1539 | 2011 | 15 | 27 | IS*481*R | *prn2* | B | *ptxP3* |
| L1540 | 2011 | 13 | 27 | IS*481*R | *prn2* | A | *ptxP3* |
| L1553 | 2011 | 13 | 27 | IS*481*R | *prn2* | A | *ptxP3* |
| L1558 | 2011 | 13 | 27 | No ISE | *prn2* | A | *ptxP3* |
| Untypeable prn, South Australia | | | | | | | |
| L1301 | 2009 | 13 | New 2 (27) | NA | Untypeable | A | *ptxP3* |
| L1304 | 2010 | 13 | 27 | NA | Untypeable | A | *ptxP3* |

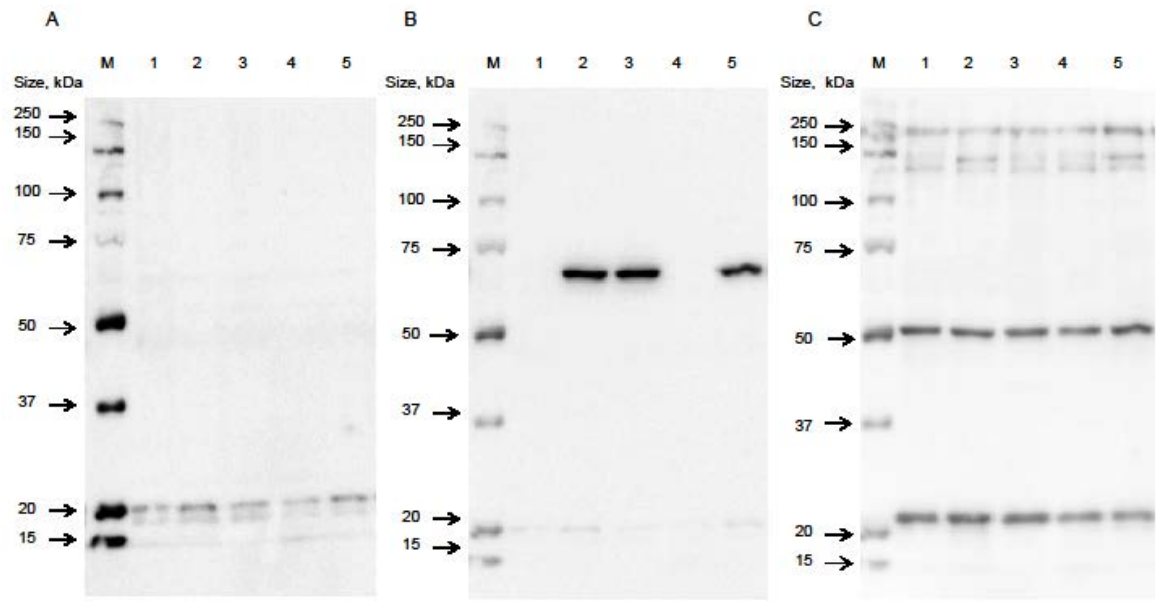
*SP,single-nucleotide polymorphism profile; MLVA, multilocus variable number tandem repeat analysis; *prn*, pertactin; *fim*, fimbrial antigen; *ptx*, pertussis toxin; ISE, IS element; NA, not applicable.

†New MLVA types identified in isolates by using the 8-loci MLVA scheme and corresponding MLVA types identified by using the 6-loci scheme are shown in parentheses.

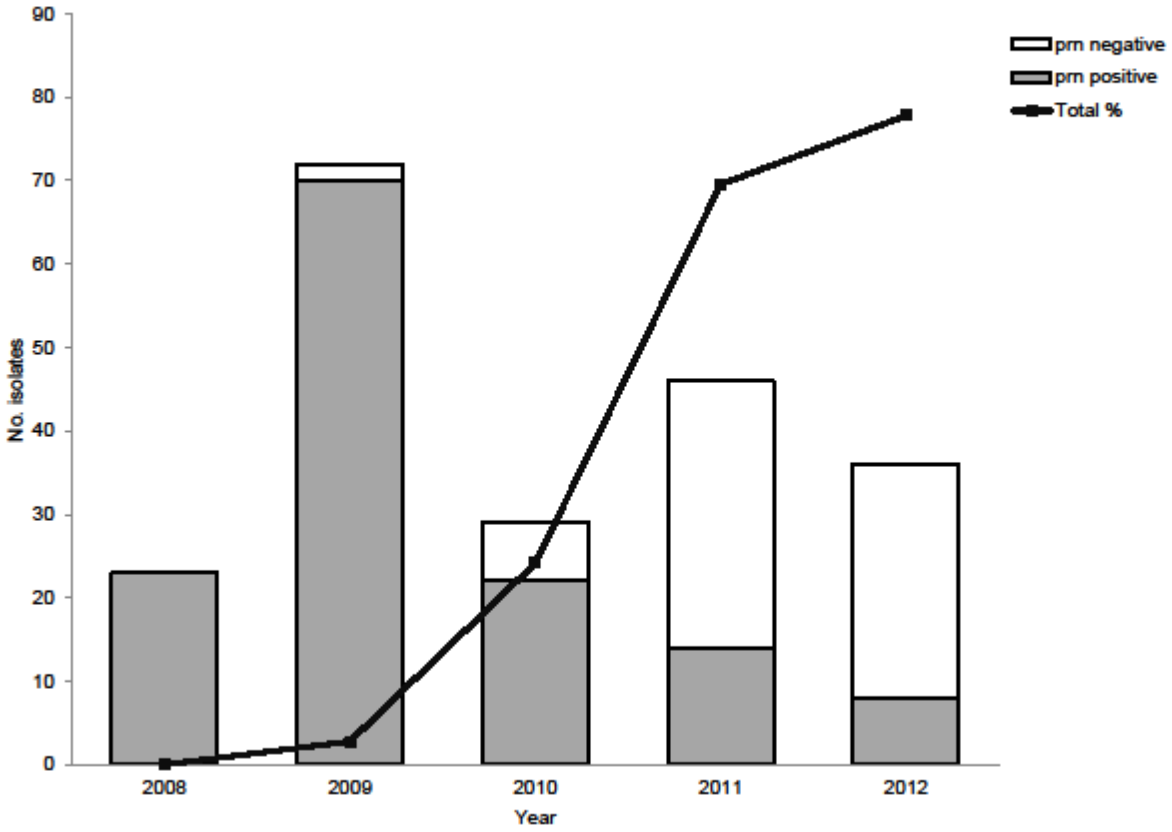
‡No ISE or other disruptions were found in *prn*; IS*481*F/IS*481*R, IS*481* inserted in the forward/reverse direction; IS*1002*, IS*1002* disruption; HPT-G, homopolymeric tract variation.




Technical Appendix Figure 1. Relationships of *Bordetella pertussis* isolates by using single-nucleotide polymorphism (SNP) typing, Australia, 2008–2012. Maximum-parsimony tree showing evolution of *B. pertussis* on the basis of 65 SNPs. Each branch is represented by a SNP profile (SP), which were grouped into clusters I–VI.




Technical Appendix Figure 2. Western blots of 5 *Bordetella pertussis* isolates treated with mouse polyclonal antibodies, Australia, 2008–2012. A) antibody against pertussis toxin (ptx), B) antibody against pertactin (prn), and C) antibody against filamentous hemagglutinin (fha). Lanes 1–5 in each panel correspond to isolates L1429, 1430, L1431, L1432, and Tohama I, respectively. Lanes M indicate molecular weight markers. All isolates showed positive results for ptx and fha. Only L1430 and L1431 expressed prn, and L1429 and L1432 did not express prn. Expected sizes are 21–23 kDa for ptx subunits, 69 kDa for prn, and 25 kDa, 50 kDa, and 125 kDa for fha.




Technical Appendix Figure 3. Number and percentage of pertactin (prn)–negative *Bordetella pertussis* isolates in 2 states of Australia (New South Wales and Western Australia), 2008–2012.